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Preclinical Models in Vascularized Composite Allotransplantation

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Abstract Vascularized composite allotransplantation (VCA) has the potential to reconstruct any non-visceral tissue defect, using like for like tissue, delivering optimal form and function. Over 150 VCA transplants have been performed worldwide; however, this treatment remains experimental. Things systematically tried in the clinic should follow thorough science and tested in a model that allows predicting as possible the safety of the procedure. This is generally the function of animal experimentation. Advantages of preclinical models include greater control of bias, of subject numbers and of variables compared to clinical trials. Limitations include differences between species in anatomy and physiology as well as ethical, logistical and technical constraints. Multiple models have been described; however, no single model is ideal in all areas. This paper reviews historical perspectives, current models and future direction in VCA research.

This article is part of the Topical Collection on *Vascularized Composite Allografts*

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Introduction

To date, over 150 VCA transplants have been performed, most commonly of the hand and face, but also abdominal wall, larynx, lower limb, uterus and penis [1, 2]. Any non-visceral tissue defect can potentially be reconstructed in this manner using like for like tissue; without donor site morbidity associated with traditional methods of surgical reconstruction. Indications for VCA include trauma of all aetiology including burns, as well as tissue loss due to infection or benign neoplasia and congenital defects [3••]. However, the patient and physician must carefully balance the risks vs. the benefits undergoing a VCA, which include, but are not limited to, surgical and immunological complications including death and side effects from immunosuppression. The latter being the most significant barrier to VCA at this time. Additionally, as a non-life saving transplant with the capacity to transform the recipient's lives, a careful patient selection cannot be overemphasised. Current reports show that most graft losses have been associated with non-compliance with immunosuppressive regimens [2]. This being said, successful hand transplants have allowed some patients to regain independence and to perform activities of daily living, whilst face transplants have enabled the isolated to lead socially integrated lives [4].

Whilst VCA is a clinical reality, it remains an experimental treatment. Thus, for the field to move forward, there is a requirement for further systematic research in all aspects of VCA. Studies based on surveys of reconstructive surgeons in the United States (US) showed that only a minority of physicians would support the practice of VCA for hand or

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face. This is mainly due the morbidity and mortality associated with systemic immunosuppression [5–7]. However, the attitude has been changing over time as results improve and strategies towards the development of tolerance, chimerism, and graft-specific treatments are being developed. It is axiomatic that no truly novel experimentation should take place on human subjects without preclinical modelling; in the opinion of the authors of this review, the current state of the art of VCA mandates further preclinical research to enable the field to move forward.

To correctly analyse these strategies, preclinical models, which can generate clinically relevant translatable results, are required. An ideal animal model would have similar anatomy and physiology, deliver reproducible and beneficial results, be inexpensive, enable the study of functional outcomes, be technically feasible and above all be ethical. Therefore, there is no single model that could encompass all of these principles. The purpose of this review is to summarise preclinical models in VCA, highlight their advantages and discuss future directions of the field.

Large or Small Animal Models

Table 1 summarises current animal models available for the study of VCA. In general terms, large animal models are anatomically and physiologically more comparative to humans and are technically easier to operate on, however they are more difficult to manage in terms of husbandry and logistics [30•]. Large animal models are better to answer questions of practicality, safety and generalised efficacy whereas small animal research is more suited to answer questions of pathway and mechanism [31]. Small animal research is typically less resource intensive enabling a larger study population, and results can be realised faster. They are easier to handle, and in the case of mice, they can be very tightly genetically controlled. However, performing microvascular surgery is very challenging leading to variability of results and difficulties in reproducibility [32•].

Historical Perspectives

Canines have been used in transplant research since the inception of the discipline, indeed the first published model of limb auto-transplantation was performed by Alexis Carrel using a dog in 1906 [33]. The first uses of immunosuppression in animal models of VCA were in canines in the 1960s and 1970s [6, 7]. There is therefore an historical body of data that enables comparative research to be undertaken. However, ethical concerns with respect to the use of companion animals in research have reduced their use. Nonhuman primate (NHP) models were developed in the 1980s and swine models at the turn of the century [12, 14]. Whilst all the species above have advantages, NHPs may be the optimum in terms of the ability to assess functional outcomes. Nonetheless, the NHP model offers very high logistic constraints associated with their use and ethical considerations reduce their applicability. At this time, swine models may offer an ideal compromise in this regard.

Tissue Type Composition

Initial immunosuppressive strategies in VCA were based on those used in solid organ transplantation, however it was found that levels of immunosuppression required in primates were much higher in VCA than in renal transplantation [14]. The heterogeneity of VCA grafts including diverse tissues such as skin, bone, muscle, tendon, nerve, vascular and lymphoid tissues is a defining characteristic and poses unique challenges as well as opportunities for discovery. The variability of allogenic response elicited by the different tissue types has been extensively studied and characterised [34–39]. The skin offers the ability to closely monitor rejection in an unprecedented way. Indeed, it is suggested that rates of rejection may be over represented compared to other solid organ transplants. It is also possible that the early management of rejection increases the long-term survival and outcomes of a skin-containing VCA. It has been reported that the bone marrow component of the VCA graft may confer immunogenic protection, and investigations including bone marrow infusions or bone marrow-derived mesenchymal stem cells have resulted in tolerance to VCA grafts, however, not without complications related to graft vs. host disease and post transplant lymphoproliferative disorders [40–43].

Nonetheless, further research is required to determine how the specificities of a VCA impact outcomes.

Heterotopic vs. Orthotopic

The location in which grafts are placed is determined by two factors, namely the technical reliability of the procedure and the safety of the position of the insensate graft, which may be at risk of self-mutilation. The benefits of heterotopic models include the optimal placement of grafts for monitoring and safety and also reduce the operative stress on the recipient animal as the requirement for an amputation is obviated. Hence, in both large and small animal models, the abdomen has been found to be advantageous, as well as the groin, neck and back [9–11, 16, 17, 19, 23].

Orthotopic models are technically more challenging, may put the graft at risk from the animal itself and may subject the animal to greater surgical stress. Benefits of this approach however are that not only can immunological factors be studied but also functional recovery of the graft, which magnifies the utility of the experiment. Technical advances to aid in performing such models have included the “cuff” technique of vascular anastomosis in small animal research to enable

Table 1 Animal models of VCA

Species			Model, author, date, citation
Canine			Hemiface (OTH)
			Bermudez 2002 [5]
			Hind limb (OTH)
			Lance 1971 [6]
			Hind limb (OTH)
			Goldwyn 1966 [7]
Swine			Forelimb (HT)
			Kiermeir 2013 [8]
			Gracilis (HT)
			Barone 2013 [9]
			Hindlimb (HT)
			Ibrahim 2013 [10]
			Hindlimb (HT)
			Hettiaratchi 2004 [11]
			Medial digital forelimb joint (OTH)
			Ustuner 2000 [12]
			Radial forelimb osteomyocutaneous flap (OTH)
			Ustuner 1998 [13]
NHP	Baboon		2nd digit
			Egerszegi 1984 [14]
			Hand (OTH)
			Egerszegi 1984 [14]
	Macaques	Rhesus	Radial side of hand and forearm (OTH)
			Hovius 1992 [15]
			1st ray (OTH)
			Stevens 1990 [15]
		Cynomolgus	Fibula (HT)
			Mundinger 2011 [16]
			Facial segment (HT)
			Silverman 2008 [17]
			Oromandibular facial segment (HT)
			Silverman 2008 [17]
			Radial forearm (OTH)
			Cendales 2006 [38]
			Mandible (OTH)
			Gold 1991 [18]
Rat			Midface (HT)
			Zor 2010 [19]
			Hindlimb (OTH)
			Kim 1984 [20]
			Hindlimb (OTH)
			Furnas 1982 [21]
			Hindlimb (OTH)
			Shapiro 1978 [22]

smaller vessels to be anastomosed [44]. Other challenges in large animal research include splinting and immobilisation of limbs that can place very large demands on laboratory staff with respect to animal husbandry and may mandate longer durations of animal sedation, with concomitant risks to the experimental subject. As an example of this balance, Sucher et al. report a significantly lower technical success rate in performing their orthotopic mouse hind limb transplant compared to their heterotopic model, a situation paralleled by our groups' experience in swine [23].

Table 1 (continued)

Species	Model, author, date, citation
Mouse	Hindlimb (HT)
	Lin 2014 [23]
	Hindlimb (OTH)
	Sucher 2010 [24]
	Hindlimb (HT)
	Tung 2001 [25]
	Hindlimb (OTH)
	Zhang 1999 [26]
	Ear (OTH)
	Jiang 1998 [27]
	Groin flap (HT)
	Cooley 1998 [28]
Rabbit	Mandible (OTH)
	He 1990 [29]

OTH orthotopic, *HT* heterotopic

Functional Recovery

The interplay between host immunological response to the graft and functional recovery of vital structures is one predictor of the value of vascularized composite allografts. For example, facial nerve regeneration, intrinsic hand muscle reinnervation, bone union or tendon healing and gliding may be required for the function of a VCA. Orthotopic models in small and large animals have been designed to address these questions. Technical success has been reported in orthotopic mouse hind limb transplantation [23, 24] and motor and sensory nerve functions have been positively evaluated in orthotopic limb transplants in both rodents and NHPs [14, 15, 22]. Nonetheless, measuring functional recovery is a challenge and various strategies have been attempted. Clinical measurements of function include the use of video gait kinematics, the Tarlov scale and electromyography, and these have been adopted for pre-clinical models. However, measuring grip strength in NHPs has been a formidable challenge [45]. Orthotopic transplantations in face transplant research have been limited to mandibular segments in rabbits and NHPs [18, 29] and a combination of nerve conduction studies of sensory and motor nerve regeneration combined with clinical analysis of functional recovery has been optimal.

Brief Immunological Considerations

Immunologic tolerance has been attempted in several species currently used in VCA research. There are strong similarities between the immune system of the human and other mammals, however it seems that the relatively longer exposure to allogens during the human lifespan causes adaptations in the immune response that are simply not present in animal species [33]. This problem is compounded by the fact that laboratory animals are typically immature when used in such investigations. Outbred animals such as canines display the most vigorous immune responses due to the relative maturity of their immune systems. The dog leukocyte antigen (DLA) system has also been well characterised due to its lengthy involvement in transplant research, however their use in preclinical studies is declining.

Swine and NHPs are typically inbred and have less mature immune systems, however swine leukocyte antigen (SLA)-typed animals are now available and offer the opportunity to test immunologic interventions in a well-controlled environment [45]. It is mandatory that immunologic differences between animals in study groups can be defined to enable the impact of experimental interventions to be accurately established.

Genetically controlled, inbred or knockout mice offer the potential for very fine control of the immune system, and increasingly feasible technical models using supermicrosurgery or non-suture-based anastomotic techniques offer the chance to gain mechanistic data in the area of VCA immunology. The mouse immune system is much more closely related to the human one in comparison to the rat [31].

Conclusions

The technical feasibility of reconstructive transplantation has achieved proof of concept and becomes a clinical reality. There remain myriad unanswered questions in the field of VCA including the relationship between rejection and function, outcomes, cost-effectiveness and chronic injury. This brief review provides a summary of the progression of

preclinical models in VCA. It is clear that no single model can answer all the questions in VCA. However, things systematically tried in the clinic should follow thorough science and tested in a model that allows predicting as possible the safety of the procedure. This is generally the function of animal experimentation.

Compliance with Ethics Guidelines

Conflict of Interest C. Anton Fries, Dmitry W. Tuder and Michael R. Davis declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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